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Abstract

Biological control of the grape, obscure and longtailed mealybugs was investigated in vineyards. Many natural enemies attack the grape and longtailed mealybugs, however, their numbers and effectiveness varies. To develop an augmentation program, methods to mass-produce parasitoids (*Pseudaphycus angelicus* and *Acerophagus notativentris*) were tested. After screening seven mealybug species (citrus, citrophilus, obscure, longtailed, striped, Comstock, and grape), results found only the grape and longtailed mealybugs were suitable insectary hosts. Of these, only longtailed is suitable for mass-production and it can be difficult to rear in large numbers. In a second project worked towards the establishment of imported natural enemies of the obscure mealybug. In 1994, a cold-hardy ″biotype″ of the ″mealybug destroyer″ was imported from Australia. This lady beetle was released in north coast (1994-96) central coast (1996-97) vineyards. Results showed that this predator overwintered in both regions, and is currently established in the central coast sites. However, its numbers have fluctuated and no economic effect on mealybug densities was found. In 1997, two encyrtid (*Pseudaphycus flavidulus* and *Leptomastix epona*) were imported from Chile and released in central and north coast vineyards. Both parasitoid species overwintered and there was a dramatic reduction in mealybug densities in 1 of 4 release blocks.

Executive Summary

The grape mealybug, *Pseudococcus maritimus*, longtailed mealybug, *Pseudococcus longispinus*, and obscure mealybug, *Pseudococcus viburni*, are part of the *Pseudococcus* "*maritimus-malacearum*" complex of closely related mealybug species. Each of these mealybug species can be a serious pest of table and wine grapes—feeding on the fruit, trunk, canes, or leaves. However, direct damage is minor because mealybug populations rarely get large enough to reduce plant vigor through feeding alone. It is the indirect damage that results in the greatest economic loss (honeydew and sooty mold accumulation, dead insects in table grape clusters). During the past decade, these mealybug species have become increasingly important pests of Central Valley table grapes (grape mealybug) and some North (obscure mealybug) and Central (obscure and longtailed) Coast wine grapes. We report on the investigation of two different control programs: augmentative release of natural enemies to suppress grape and longtailed mealybugs, and classical biological control—to control obscure mealybugs.

The grape and longtailed mealybugs are attacked by many parasitoid species believed to be native to North Ameri In fact, resident natural enemies most often control these two mealybug species. However, recent surveys of mealybug populations indicate that parasitoid activity can vary considerably among vineyards and, when parasitoid activity is low, mealybug infestations typically increase and cause economic damage. It is not clear why parasitoid populations drop to low levels; however, it is clear that their effectiveness is reduced considerably. For this reason, augmentation of parasitoids may be used to improve mealybug control and lessen the reliance on synthetic insecticides. Augmentation of natural enemies of mealybugs has been used successfully in other countries. Further, augmentation is compatible with all aspects of IPM pest control strategies and sustainable farming practices. This research investigated the feasibility of augmenting two encyrtid

parasitoids (*Pseudaphycus angelicus* and *Acerophagus notativentris*) and one cecidomyiid predator of grape and longtailed mealybugs to increase parasitism levels and reduce the need for insecticide applications.

For the successful development of a cooperative insectary, economic rearing methods must be developed to produce high quality parasitoids. In 1997 and 1998 we screened seven mealybug species: citrus (*Planococcus citri* [Risso]), citrophilus (*Pseudococcus calceolariae* [Maskell]), obscure, longtailed, striped (*Ferrisia virgata* [Cockerell]), Comstock (*Pseudococcus comstocki* Kuwana), and grape mealybug—as potential insectary hosts for *A. notativentris* and *P. angelicus*. Only the grape and longtailed mealybugs were suitable as hosts for these parasitoids. Of these two mealybug species, only the longtailed mealybug is suitable for mass-production and even it can be difficult to rear in large numbers.

To date, the following plant hosts have been tested (gravid female mealybugs were placed on the plant and their offspring development and survival followed): grapevine cuttings potted in 1-gallon containers, squash (acorn squash, butternut squash, Kabocha or ?Japanese pumpkin″), iceplant, 4 potato varieties, and ornamental plants (*Dracaena*, pothos ivy, African violet, croton, and philodendron). Sprouted potatoes and squash appear to be the most cost-effective (cost to the number of mealybugs produced) host plants to rear longtailed mealybug. No host plant tested has yet been found to mass-rear grape mealybug for parasitoid production. Production of longtailed mealybug colonies has been poor because of their slow development and low fecundity on ″non-grape″ host plants. The lack of effective rearing procedures has delayed the experimental release of *A. notativentris* and *P. angelicus* in field tests.

In a second project, research investigated the establishment of imported natural enemies of the obscure mealybug. Unlike the grape mealybug, the obscure mealybug is probably not native to North America and there are no resident parasitoids that specialize on this pest. In 1997, two encyrtid parasitoids (*Pseudaphycus flavidulus* and *Leptomastix epona*) were imported from Chile. These parasitoids were reared in the insectary and released in the central coast and Carneros region vineyards. Field samples collected in 1998 indicate that both species overwintered. While sampled vineyards still have relatively high mealybug densities, there is evidence of good parasitoid activity.

In 1994, K. S. Hagen imported a "biotype" of the mealybug destroyer, *Cryptolaemus montrouzieri*, which may be better adapted to cold weather. This beetle was released in Carneros region in 1995. In the 1995-97 seasons, we made recoveries in the release area and noted a considerable increase in *C. montrouzieri* numbers, providing evidence that it has established in California. However, during the 1998 season there was a mealybug pest problem, and we have not yet recovered any beetles. In the 1997-98 season, we concentrated releases in central coast vineyards and recovered *C. montrouzieri* in spring (1998) before new releases were made—indicating that this predator successfully overwintered.

In conjunction with the natural enemy release studies, we investigated the interaction between ants, mealybugs, and the imported natural enemies. For this work, we established ant-exclusion and no-exclusion field plots, conducted laboratory trials, and produced an 18-minute grower video.

Conclusions from this work are definitive: ants tending mealybugs milk them for honeydew and attempt to protect them from predators and parasitoids. In the small video arena, the ants were often successful in disrupting parasitoid oviposition. Ants were less successful in capturing the mealybug destroyer, which has physically and behaviorally mimicked the mealybug. In the field studies, data (still being collected) indicate that mealybug densities are lower in the ant-excluded treatment; however, parasitoids have been recovered from both treatments (indicating that parasitoids attack the mealybug even in the presence of foraging ants).

Introduction

Mealybug Pest Status. There are four mealybug species that cause economic damage in North American vineyards. These are the grape mealybug, *Pseudococcus maritimus* (Ehrhorn), obscure mealybug, *Pseudococcus viburni* (Signoret), the longtailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzeti); and the vine mealybug, *Planococcus ficus* Signoret. Three of these species (obscure, longtailed, and grape) belong to the *Pseudococcus maritimus-malacearum* complex - a group of closely related mealybugs that overlap in host ranges and natural enemies (Wilkey & McKenzie, 1961). Economic losses resulting from this pest complex have mounted dramatically in the past decade. The grape mealybug has become a primary pest of California's table grape industry (Daane *et al.* 1996, Geiger *et al*. 1999). The obscure mealybug has surfaced as a primary pest of some central coast vineyards (Daane *et al.* 1996) and has recently been identified as the mealybug species causing considerable damage to north coast vineyards in the Carneros region. Although the longtailed mealybug is one of the most widely cited pest management problems for interior plantscapes (e.g., shopping malls) (NCCES, 1997), it is also a sporadic but important pest in some central coast vineyards.

Mealybugs in the *P. maritimus-malacearum* complex can feed on the grapevine's fruit, trunk, canes, or leaves. Severe mealybug infestations result in late-season defoliation in vineyards and in some stone fruit. However, mealybug densities rarely get high enough to reduce plant vigor directly through feeding alone. It is the indirect damage that often results in the greatest economic loss. As mealybugs feed they excrete the unused plant sap (honeydew), which promotes sooty mold (fungi) growth on the leaves and fruit. Live and dead mealybugs (or their cottony wax secretion) also accumulate on the plant (and fruit). The honeydew, sooty molds, and insect parts are unsightly on ornamental plants and lower fruit marketability in agricultural crops.

Currently, the most effective control is a late-dormant insecticide application [(typically Lorsban) (Bentley *et al.*, 1997)], which is dependent on a Section 18 exemption to use dormant oil sprays with Lorsban. As a result, mealybug control is now on the USDA list of California IPM priorities.

Although mealybugs infest a relatively small portion of table and wine vineyards, when present they can be devastating. Insecticides do not provide consistent control and often have additional problems of environmental contamination, secondary pest outbreaks, or legislative restrictions. The proposed studies will provide the needed biological information to improve the IPM of grape and obscure mealybugs.

Augmentation Studies. For the grape and longtailed mealybugs, we sought to develop augmentative biological control programs. Both mealybug species are good candidates for such programs. There are, in fact, many examples of both grape and longtailed mealybug populations suppressed naturally by the action of parasitoids. In most agroecosystems, there is a complex of parasitoids and, of these, there are often overlaps in host ranges (Table 1). Clausen (1924) recorded >80% parasitism of grape mealybug in the San Joaquin Valley, with five species reported as common and with *Z. corvinus* (Girault) the dominant parasitoid. In recent surveys there were considerably lower and more variable parasitism rates (0-70%) (Daane *et al*., 1996). There was also an apparent shift in parasitoid species composition — the dominant parasitoid species in recent collections were *A. notativentris*, and *P. angelicus*, with *Z. corvinus* rarely collected. Similarly, early surveys of the longtailed mealybug also showed a diverse assemblage of parasitoids (Noyes & Hayat, 1994), while recent surveys of longtailed mealybugs in California's central coast vineyards found only three parasitoid species (*P. angelicus*, *Encyrtus* sp. and *Zarhopalus* nr. sp. *sheldoni*) (<10%) (Daane *et al.*, unpublished. data).

Augmentative release may help to re-establish effective parasitism levels in these vineyards. The critical biological information needed to develop a successful augmentation program includes selection of mealybug species for insectary rearing. Based on preliminary research, we have selected longtailed, grape, Comstock, and citrophilus mealybugs as potential insectary hosts. Based on field-collections of longtailed and grape mealybugs (Daane *et al*. 1996), we have selected four encyrtid parasitoids as candidates for augmentation: *Pseudaphycus angelicus* (Howard), *Acerophagus notativentris* (Girault), *Zarhopalus corvinus* (Girault), and *Zarhopalus sheldoni* Ashmead. Laboratory studies have begun which investigate mealybug and parasitoid biology to address questions concerning insectary methodology and parasitoid suitability (e.g., temperature requirements, development rate, fecundity, and parasitoid vigor).

Classical Biological Control. The second project is directed towards the release, establishment and evaluation of obscure mealybug natural enemies. Prior to 1993, no parasitoids were reared from obscure mealybugs collected in California vineyards (Daane, unpubl. data). In fact, it was the lack of parasitoid activity on grape mealybugs collected from pear trees that led taxonomists to suspect that mealybugs grouped as the "grape mealybug" were, in fact, a complex of two or more species. Maskell first described the obscure mealybug in 1894. Many obscure mealybug specimens collected on agricultural crops prior to 1960 were misidentified as the grape mealybug or some other species. McKenzie (1967) lists *P. obscurus* Essig, *P. capensis* Brain, *P. maritimus*, *P. malacearum* Ferris, and *P. longispinus* as synonyms or misidentifications of the obscure mealybug. Wilkey & McKenzie (1961) and Miller *et al*. (1984) found diagnostic characters that provided the needed taxonomic descriptions of the *"maritimus-malacearu"* complex to enable researchers to

properly identify species and better describe their geographic range and host plants. Once these species were properly separated, it was discovered that an effective parasitoid complex attacking the obscure mealybug was lacking.

The large majority of successful mealybug biological control efforts have used "encyrtid" parasitoids (Greathead 1986). For this reason, we initiated an importation program in 1996 and, in 1997, S.V. Triapitsyn and K. M. Daane traveled to the table grape region of Chile and searched for obscure mealybug natural enemies. Working in collaboration with Chilean researchers, *Pseudaphycus flavidulus* and *Leptomastix epona* (Walker) were imported. The parasitoid material was processed through quarantine, released to the University of California insectary, and mass-reared for field release. We report here on efforts to establish these parasitoids and determine their economic impact.

Another part of the research program concerns the well-known lady beetle, the mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant. The mealybug destroyer is, perhaps, the most effective mealybug predator; unfortunately, the beetle was imported from relatively warm regions in Australia and this "biotype" cannot overwinter in the colder regions of California (Bartlett 1978). In 1994, K. S. Hagen imported a "biotype" of this beetle, which may be better adapted to cold weather. This beetle was released in the central coast and Carneros regions, and in the 1995-96 season it successfully overwintered in the Carneros region, providing evidence that it has established in California. In the 1996 and 1997 seasons, the mealybug destroyer was found in significant numbers in Domaine Chandon vineyards (and we suspect that it contributed to a reduction in mealybug numbers). Here, we report on the release and potential establishment of this important predator in north coast and central coast vineyards (in 1996-99).

Materials and Methods

Objective 1. Investigate the potential of rearing two parasitoids (*Acerophagus notativentris* and *Pseudaphycus angelicus*) and a cecidomyiid midge for control of grape and longtailed mealybugs.

a) Test the grape and longtailed mealybug as an insectary host for both parasitoid species (including tests for environmental stimuli that could accelerate mealybug population growth).

Grape mealybug and longtailed mealybug were reared on a variety of media (see #1C below). Mealybugs on sprouting potatoes were placed in 1-quart glass jars with paper towels and a muslin lid. Parasitoids were introduced in various quantities depending on availability. Parasitoid species tested were *Acerophagus notativentris*, *Pseudaphycus angelicus*, *Leptomastix epona*, and *Zarhopalus corvinus*.

Colonies were separated to avoid contamination, making use of UC Berkeley insectary rooms. The ease of insectary production of each mealybug species on potatoes was observed. Potatoes from citrus, citrophilus, grape, longtailed, and obscure colonies were selected that had about 50 mealybugs of various development stages present. Infested potatoes were placed, individually in Dixie cups where female and male parasitoids were added. After 21 days, the potatoes were examined and the number of mealybugs and mealybug mummies were counted.

b) Evaluate the quality and quantity of parasitoids produced by longtailed mealybug.

Eight to twelve individuals of *P. angelicus* were placed in glass jars containing longtailed mealybug from colonies. These were held at room temperature and observed daily for emergence of second-generation parasitoids.

c) Test new plant host material to rear the grape, obscure and longtailed mealybugs.

Potatoes of several varieties were tested, using several sprouting techniques. Varieties tested were Russett Burbank, Norkotah, and Red Lasota. All were sprouted in the dark at approximately 21°C. Some were sprouted without substrate, some were placed in moist sand in trays, and some were placed in buckets with moist sand with eyes down. Hoagland's solution (diluted to 1/4 strength) was used to water sprouting potatoes to prevent calcium deficiency, which can cause potato sprouts to wither prematurely. An informal experiment was also conducted to compare the effects of rooting hormone (Rootone) and intensive scrubbing of potato skins sprout size and growth.

Cuttings from grape vines were also tested for rearing the grape mealybug. In this technique, sections of grape cane approximately 0.3-0.5 m. long were scored with a knife to expose strips of phloem tissue. The lower end of the canes were placed in moist rock wool to promote rooting, and the rest of the canes were loosely enclosed in strips of paper towels to simulate bark.

These canes were placed horizontally on top of field-collected spurs with emerging crawlers. Sprouting potatoes, fresh bouquets of grape leaves, and bouquets of *Pithosporum undulatum* leaves were also placed in the same box for comparison of crawler settling behavior. Once the crawlers settled, the canes were planted in pots in a greenhouse.

Field-collected grape mealybug ovisacs were also placed on squash (acorn squash, butternut squash, Kabocha or "Japanese pumpkin"), iceplant, sea fig, and young apple trees. Longtailed mealybugs were tested on the above squash varieties and several ornamental plants (*Dracaena*, pothos ivy, African violet, croton, and philodendron).

d) Identify species of cecidomyiid midges found in grape vineyards and determine the feasibility of mass-production.

Cecidomyiid larvae were collected in bark samples from Kern County vineyards. They were then placed on potatoes infested with the citrus mealybug, *Planococcus citri* (Risso), in a sleeve cage. More mealybugs were provided as needed to maintain the culture. Adult midges were mailed to Dr. Raymond Gagne, USDA, for positive identification.

Objective 2. Determine the effect of inoculative release of *A. notativentris, P. angelicus*.

Parasitoid production was not sufficient to test inoculative releases in the field.

Objective 3. Release imported natural enemies against the obscure mealybug and continue to measure the effect of natural enemies in Central and North Coast vineyards.

a) Release and evaluate the parasitoids *Pseudaphycus flavidulus* and *Leptomastix epona* for control of obscure mealybug.

b) Release and evaluate a "cold-hardy" strain of the mealybug destroyer.

In February1997, KMD, Dr. Gonzalez and Dr. Triapitsyn (UC Riverside) searched for natural enemies of the obscure and vine mealybugs in Chile and Argentina. We also observed the insectary operations in Leon (Chile), where obscure mealybug natural enemies are mass-produced for release in Chilean vineyards. From Chile, we imported *P. flavidulus* and *L. epona.* In spring 1997, this material was processed in the UC Berkeley quarantine. In summer 1997, the parasitoids were released from quarantine and mass-produced in the UC insectary. In 1997-99, *P. flavidulus* and *L. epona* were mass-produced on the obscure mealybug reared on sprouted potatoes. During this period, 50,000 *P. flavidulus* and 10,000 *L. epona* were produced. In July, August, and September of 1997, 3,200 *P. flavidulus* and 1,500 *L. epona* were released in San Luis Obispo and Santa Barbara Counties. From summer 1998 to winter 1998/99, 20,000 *P. flavidulus* and 4,000 *L. epona* were released at three Central Coast sites: Paragon (San Luis Obispo Co.), MacGregor (San Luis Obispo Co.), and White Hall (Beringer) (Santa Barbara Co.) vineyards; and 5,000 *P. flavidulus* and 2,000 *L. epona* were released in the Carneros region: Domaine Chandon and Buena Vista Vineyards (Napa Co.).

Production of the mealybug destroyer was accomplished on citrus mealybug reared on sprouted potatoes. From 1995-96, 5,000 adult beetles were released in the Carneros Region (prior to DPR funding). In 1997, releases in the Carneros regions were discontinued in order to evaluate permanent establishment and effectiveness of the beetles. In 1997, 2,500 adult mealybug destroyers were released in two Central Coast vineyards (Paragon and MacGregor).

In 1998, two different sample methods were used to measure natural enemy establishment and impact. First, during the growing season (April through November) unmarked vines near the release site were searched for mealybug mummies (parasitoid presence) and beetle larvae. All

mummies and beetle larvae collected were taken to the laboratory, reared, and the resulting adults were identified. This "gross" sampling method allowed us to search 1,000s of mealybugs for signs that the released natural enemies were reproducing in the vineyards and to determine which species were present.

The second sampling program was coordinated with a study of ant interactions with released parasitoids (see appendix). Three vineyards were used for the ant exclusion trials: a wine vineyard near, Napa, CA (Domaine Chandon, Chardonnay cultivar) and 2 wine grape vineyards near San Luis Obispo, CA (Paragon and MacGregor vineyards, Chardonnay cultivar). *Pseudaphycus flavidulus* and *L. epona* were released in all winegrape sites. At each site, 60 to 70 vines, which had ants actively tending mealybugs, where selected from the larger block. The Domaine Chandon block was 5 rows by 75 vines, while the Paragon and MacGregor blocks were 12 rows by 30 vines. In each block, 5-vine plots were established and treatments (ants or no ants) were assigned in either a completely random (Domaine Chandon only) or randomized block design. To exclude ants, the basal 2 to 3 inches of the vine trunk and post were cleaned and wrapped with duct tape, which was then coated with Tanglefoot (a sticky, semi-solid barrier). To prevent aboveground movement between treatments, a 1-foot section of grape foliage (canes and leaves) was cleared between each treatment and the exposed trellis wires and irrigation lines (for drip irrigation systems) were coated with a 2 to 3 inch barrier of Tanglefoot.

To begin sampling, one half of one vine was randomly selected from each plot. A visual count of ants on sample vines was made: 30 seconds for the exclusion (no ant) treatment (to confirm no ants had broken through the barrier) and a 2 minute count of ants moving up or down the inner cordon on the non-exclusion (ant-tending) treatment. A destructive sample was then taken. A 150 cm^2 sub-sample of the trunk was taken on the inner cordon or upper trunk, with the number, development stage and condition of mealybugs recorded. On spurs 1, 3, and 5 (moving from the trunk) all bark around the spur (at 3.5 mm above and below the cordon-joint) was removed and examined for mealybugs. Seven basal leaves each from the sampled spurs were examined in the field and mealybug abundance, development stage and condition were recorded. Three grape clusters were collected on canes originating from each of the sampled spurs. The clusters were placed in plastic bags, stored at 15°C, and later dissected in the laboratory.

On each sampled section the abundance, development stage, and condition of mealybugs were recorded (e.g., adults, second-third instar mealybugs, new ovisacs with and without eggs, new ovisacs with crawlers, parasitized mealybugs). Since crawlers were often too numerous to accurately count, only their presence or absence was noted initially. A rough scale was used later (e.g., 0-10, 10-20, 30-40) to count crawlers not in an ovisac. Also noted were predators (e.g., beetles and lacewings). All new mummies (those from which parasitoids had not yet emerged) were collected, placed in glass vials and held for parasitoid emergence. Samples were taken monthly during the growing season and bimonthly during the dormant season.

Results

Objective 1. Investigate the potential of rearing two parasitoids (*Acerophagus notativentris* and *Pseudaphycus angelicus*) and a cecidomyiid midge for control of grape and longtailed mealybugs.

a) Test the grape and longtailed mealybug as an insectary host for both parasitoid species (including tests for environmental stimuli that could accelerate mealybug population growth).

Both *A. notativentris* and *P. angelicus* colonies performed best on grape mealybug hosts; however, grape mealybug remained a difficult species to rear in quantity (see #1C below). *P. angelicus* successfully reproduced on longtailed mealybug, but this mealybug species appears to have a relatively low fecundity. If factors inducing dormancy/diapause can be identified, grape mealybug and longtailed mealybug remain the best option for mass rearing *P. angelicus.*

Our screening trials tested the two parasitoid species with four alternate mealybug hosts: the obscure, citrus, citrophilus, and striped mealybugs. None of these were satisfactory hosts for *P. angelicus* or *A. notativentris*, although all are easily produced in the insectary. Currently, two other mealybug species are being testes as potential alternate hosts: the Comstock and the Mexican mealybug, *Pseudococcus madeirensis* Green. Both of these species are relatively easy to rear on potatoes, although the presence of the highly effective parasitoids *Pseudaphycus malinus* Gahan (Encyrtidae) and *Allotropa burrelli* Muesebeck (Platygasteridae) in California slows the development of clean Comstock mealybug cultures. The Comstock mealybug is known to be a host for *Zarhopalus corvinus* (Girault) (Encyrtidae), a solitary parasitoid of grape mealybug that was the most important parasitoid species in early surveys. A few *Z. corvinus* were recovered from initial field collections of Comstock mealybug. Comstock is also suspected to be a host to *A. notativentris,* but our mealybug colony has not yet reached sufficient size to conduct a screening test. We began our colony of *P. madeirensis* in spring 1999, and will screen the species as an alternate host for *P. angelicus* and *A. notativentris* in the coming months.

Experiments to measure lethal lower temperature and to test for the existence of cold-induced diapause were begun in late 1998. These experiments could not be completed due to the decline of the grape mealybug colonies. Similar experiments conducted using the obscure mealybug found that lowering the temperature to 10°C for one or two weeks resulted in slightly more synchronized egg hatch. No evidence of a true cold-induced diapause has yet been found for this species.

b) Evaluate the quality and quantity of parasitoids produced by longtailed mealybug.

It was found that the longtailed mealybug can be used as an insectary host for *P. angelicus*. *P. angelicus* reared on longtailed mealybug appear slightly smaller than those reared on grape mealybug, but this difference has not yet been quantified due to inadequate stock. Production of longtailed mealybug was more difficult than citrus or citrophilus, due to:

(1) A slow development time (~5 weeks for the citrus mealybug and ~10 weeks for the longtailed mealybug at ~80°F).
(2) The periodic dormancy of reproductive female mealybugs.
(3) Much lower production of larvae per female mealybug, as compared with the citrus or citrophilus. Nevertheless, a local insectary facility has reported success in developing mass-rearing techniques for longtailed mealybug, which may allow for the mass-production of *P. angelicus*.

c) Test new plant host material to rear the grape, obscure and longtailed mealybugs.

Grape mealybug colonies were started in the spring of 1998 and have been maintained since that time. The colonies have been reared most successfully on fertilized, watered potato sprouts in plastic buckets, using the Red Lasota potato variety. Potatoes must be carefully sorted before sprouting to minimize fungus infections, which affect red potato varieties especially quickly. The addition of fertilizer appeared to prevent calcium deficiency and wilting of sprouts, but may lead to an increase in fungus problems. A solution of calcium sulfate is now being tried as an alternative supplement. The ideal temperature for both grape, longtailed and obscure mealybugs is 21-24°C.

Grape mealybugs have also been successfully reared the prepared pieces of grape cane. Initial growth on grape canes appears to be faster than on potatoes, so this technique holds some promise. Mealybugs also successfully established on young apple trees, but failed to reproduce on squash, ice plant or sea fig, although obscure mealybug did very well on these plants. It is likely that literature records of grape mealybug on ice plant actually referred to obscure mealybug, since these species were confused for over 50 years.

Grape mealybug crawlers did not settle easily on potatoes. In experiments with crawler settling behavior, hundreds of crawlers settled on two bouquets of fresh grape leaves, 50 settled on six grape canes, less than 10 crawlers settled on two large sprouted potatoes in the same box, and no crawlers settled on *Pithosporum* leaves. Cotton wool was wrapped loosely around the potato sprouts to satisfy the mealybugs' thigmotaxis, but this seemed to have no effect. It is possible that there is a volatile chemical in grapes required for host acceptance by grape mealybug crawlers. We will conduct simple experiments with crude grape extracts in the coming months to test for such a chemical cue.

The grape mealybug colonies on sprouted potatoes declined in vigor after one or two generations. The cause is not known, but a host-quality related diapause or dormancy mechanism is suspected. It is not yet known whether a similar decline occurs in mealybugs reared on grape vines or apple

trees. Although rearing mealybugs on grapevines in a greenhouse is considerably less convenient than rearing them on potatoes, the advantages may outweigh the costs, particularly if small pieces of cane (rather than full-sized vines) can be used as media. We are currently experimenting with the use of prepared grape canes as a rearing medium.

Like grape mealybug, the longtailed mealybug was reared most successfully on sprouted red potatoes in buckets, and also appeared to be subject to a wintertime dormancy or diapause. Populations introduced to squash or potted plants were less successful, contrary to the findings of some other researchers. The plants were housed under artificial lighting indoors at about 27°C, which may help account for the poor performance. Longtailed mealybug colonies seemed to improve their performance considerably at slightly cooler temperatures of 21-23°C.

d) Identify species of cecidomyiid midges found in grape vineyards and determine the feasibility of mass-production.

The midge species commonly found in Kern County vineyards was determined to be *Dicrodiplosis californica* felt, a species originally described from a *Pseudococcus* sp. on *Solanum* sp. in Riverside. In culture with citrus mealybug, the field collected larvae matured, emerged as adults, and produced a second generation. The second generation, however, did not reproduce despite an abundance of prey. While additional experimentation might reveal a workable rearing system for this predator, *D. Californica* does not show immediate potential for mass-rearing and release programs.

Objective 2. Determine the effect of inoculative release of *A. notativentris, P. angelicus*.

Parasitoid production was not sufficient within the time period allowed to permit inoculative trials.

Objective 3. Release imported natural enemies against the obscure mealybug and continue to measure the effect of natural enemies in Central Coast vineyards and in Carneros region vineyards.

a) Imported and release the parasitoids *Pseudaphycus flavidulus* and *Leptomastix epona* for control of obscure mealybug.

b) Release and evaluate a "cold-hardy" strain of the mealybug destroyer.

Collections made after release in winter and spring of 1998 and spring of 1999 recovered both *P. flavidulus* and *L. epona* from all release sites in the Central Coast and many more mealybug mummies with emergence holes were found. At 3 of the 4 release sites there was not a noticeable reduction in mealybug densities. However, at one release block (Paragon vineyards, Chardonnay) the mealybug population density decreased dramatically, from an 1997 infestation rate (at harvest) of 40% of the bunches with mealybug to a 1998 infestation rate of <2% of the bunches. We suspected an insecticide was applied, however, the grower collaborator insists that no chemicals

were used and credits the parasitoids with some to all of the pest decline. Parasitism levels remain <10% at all sites and, to date, at only one block of one vineyard (Paragon) was there mealybug control. Levels of parasitoid effectiveness may be difficult to correctly assess because of the presence of ants (we have observed ants removing mealybug mummies). To date, no parasitized mealybugs have been found at the Carneros release site.

In 1994, Dr. Hagen imported a cold-hardy strain of the mealybug destroyer, which was mass-produced and released in Carneros-region vineyards (Napa and Sonoma Counties. Over the next two years, the ratio of mealybug destroyer to adult mealybugs increased. Before the inoculative release, no mealybug destroyers were found at the release sites; in 1995, 1 beetle was found for every 1,200 mealybugs collected in grape bunches (at harvest) and by 1996 there was 1 beetle per 100 mature mealybugs. Other lady beetles feeding on mealybugs were found. *Hyperaspis* nr. sp. *lateralis* and *Scymnus* sp. are small (~0.1 inch) lady beetles with larvae that have long, waxy filaments and superficially resemble a mealybug; the adult is shiny black with yellow spots on its back-side (the hardened wings or "elytra").

Releases of the mealybug destroyer were discontinued in 1997 at the Napa sites to determine if this predator had established. There were no beetles recovered from samples collected release sites and at the ant-exclusion site in 1997 and 1998. This followed two unusual years for weather: in winter 1997/98 and spring 1998 "El-Nino" rains pounded the northern grape growing region, leaving some vineyards underwater (although not at the Domaine Chandon site), and the winter of 1998/99 brought extremely cold weather to all of California. We believe this change in climate, two years of unseasonably harsh winter conditions, brought an end to three years of establishment of the cold-hardy strain of the mealybug destroyer in the Napa Valley region. In contrast, recoveries of this beetle were made in spring at both the Paragon and MacGregor sites in 1997 and 1998—indicating overwintering survival of the beetle (there releases in the summer and fall of both years).

Discussion

Grape mealybug has been particularly difficult to rear for more than one or two generations. These results are similar with those obtained by other researchers and insectary managers. Like other researchers, we experienced booms and crashes of grape mealybug populations. We suspect that the grape mealybug (and possibly the longtailed mealybug) is subject to a diapause or dormancy of some kind, possibly activated by a decline in host quality. Contamination of grape mealybug cultures with obscure mealybug was also a problem, and despite previous determinations we believe it is possible that some of the San Joaquin Valley areas may have a mix of the two species. In spring, 1999, it was necessary to replenish grape mealybug stocks again from field collected material. Because a reliable supply of mealybugs was essential for conducting some of the proposed experiments (parasitoid biology and field-release trials), some of the work was not completed (Objective 2).

There is still great promise of insectary production of one grape mealybug parasitoid (*P. angelicus*) on longtailed mealybug. While this insect remains the "best" alternative host, production of large numbers of longtailed mealybug is far more difficult than citrus, citrophilus, striped, obscure or Comstock. Future studies will investigate alternate parasitoid species - which can be reared on mealybugs more suitable for insectary production but are not the common parasitoids in the field. Release of *Pseudaphycus flavidulus* and *Leptomastix epona* is on schedule and continues to be very promising. The insectary production methods have been improved and we have plans to mass-rear >150,000 P. flavidulus and 50,000 L. epona in 1999. More exciting, at each release site there was a recovery of both parasitoid species. There are some questions about ant interference (see Appendix 1). In 1997, the cold-hardy strain of the mealybug destroyer was released in Central Coast vineyards. Winter temperature in this region (between San Luis Obispo and Santa Barbara) did not drop to levels as low as in the Napa region. Samples collected in the spring and summer of both 1998 and 1999 produced mealybug destroyer. As there were no releases of this predator in these fields since 1997, these results indicate that the beetle successfully established and survived two harsh winter periods. The effective of this beetle on obscure mealybug densities (in Central Coast locations) was low and it does not appear to have as much promise (to control obscure mealybugs) as the parasitoids. Further research should be completed on the biology of natural populations of the obscure mealybug and the mealybug destroyer to determine the synchrony of the egg laying periods of both pest and beneficial insects. For maximum effectiveness, the adult beetle needs to have egg sacs of the mealybug present, to both increase fecundity and provide a site for egg-deposition.

Summary and Conclusions

Insectary production methods for two encyrtid parasitoids (*Pseudaphycus angelicus* and *Acerophagus notativentris*) were tested. The investigation has not yet found a reliable system of host plant and mealybug species that can economically be used to mass-produce parasitoids. However, results are valuable as methods have been perfected for a number of mealybug species and research continues ton the longtailed mealybug as a potential alternative. The importation and release of two encyrtid parasitoids (*Pseudaphycus flavidulus and Leptomastix epona*) against the obscure mealybug has produced promising results. Insectary methods have been perfected to mass-rear these parasitoids on obscure mealybugs (on sprouted potatoes) and releases were made in central and north coast vineyards. Similarly, a "cold-hardy" strain of the mealybug destroyer was imported from Australia, mass-reared and released in central and north coast vineyards. Recoveries of each species were made. The encyrtid parasitoids show the greatest promise for natural control.

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Note: other manuscripts are in progress or planned.

Appendix 1.: Ant Interactions With Natural Enemies.

Work in the vineyards with imported parasitoids showed the importance of ant control for improved parasitoid effectiveness. These observation led to studies of the effect of ants on *Pseudaphycus flavidulus* and *Leptomastix epona*. Note: this work was funded by a grant from the California table Grape Commission and is presented in their annual report. We present here exerts from that report - as stated, this research was directly connected to this currently funded DPR research. We note here that we have received a grant from the American Vineyard Foundation to continue the insectary work with obscure mealybug parasitoids. This award was based on research conducted with support by the current DPR grant.

Part I. Video production: An 18-minute video was produced to provide growers with a detailed description of ant/natural enemy interactions. To produce the video, insectary colonies of the following ants, mealybugs and natural enemies were maintained. Obscure (*Pseudococcus viburni*) and grape mealybugs were reared on sprouted potatoes. Native gray ant (*Formica aerata*) were reared as a colony housed in 8 gallon plastic tubs. The tubs were filled with dirt, for the colony structure, and ringed with Tanglefoot to prevent ants from escaping. Dead navel orangeworm (*Amyelois transitella*) pupae were supplied as a protein support, and 25% water-diluted sugar was supplied as a carbohydrate source. Ants were trained to forage through a 3 foot long, ? inch diameter plastic tube for food. After initial training, the tube end could be position to any food source to manipulate "natural" ant foraging behavior. The mealybug destroyer (*Cryptolaemus montrouzieri*), and parasitoids (*Pseudaphycus flavidulus* and *Leptomastix epona*) were reared on mealybugs in the UC Berkeley and KAC insectaries. Green lacewings (*Chrysoperla carnea*) were field collected.

A test arena was designed with small mealybug populations on potted grapevines and a housing unit to enclose parasitoids in the test arena. The ants were supplied to the arena through the foraging tube, which could be used to manipulate ant foraging onto specific area of the plant. The 3-sided aquarium allowed for easy observation and movement of the video camera close to the mealybugs and foraging ants.

Interactions between ants, mealybugs and natural enemies were recorded with a COHU High Performance Color Camera placed on a Zoom or a Microscope and connected to a Video Cassette Recorder (Sony Hi8) (courtesy of Dr. Beth Grafton-Cardwell). For each interaction between ants and natural enemies, predators or parasites were added to the system and filming continue until their natural foraging behavior brought mealybug natural enemies to ants.

Over 3 hours of ant-mealybug-parasite interactions were recorded with close-up photography. The video has been edited to ~18 minutes and divided into 4 sections: (1) mealybug biology, (2) ant-mealybug interactions, (3) natural enemy biology, and (4) ant-natural enemy interactions. The video is available (with KMD) for presentation to groups of 10 or more growers. In 1999, we will make slight improvements to he tape, including a copy with a narration.

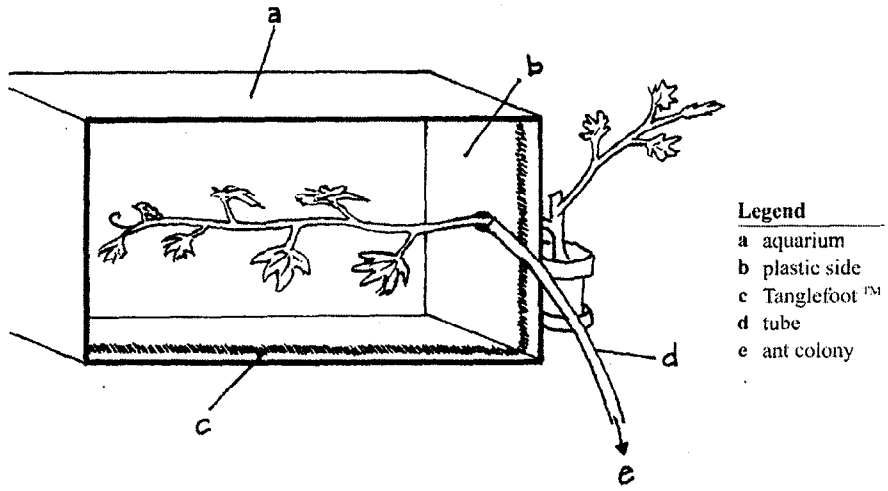

Figure 1.

Highlights from this video are:

- Mealybug crawlers are very mobile, and while later stages can move (even gravid adult females), they are quite sedentary once they establish a feeding site. Mealybugs will group together, even when released as individuals.
- The eggs take about 7 to 10 days to hatch. After the pale-yellow to orange colored crawlers find a feeding site and become more sedentary, they start excreting the white wax, which helps protect them from predators.
- Honeydew production was first observed at the second instar stage. We also note that the honeydew does not drop but is propelled away from the mealybug.
- In the laboratory arena, there were at least 7 and at most 15 ants always present with each large grouping of mealybugs. Ants appeared to take turns tending the mealybugs. If there was no honeydew deposited on the leaf, ants were able to solicit the mealybugs with their antennae, touching them repetitively on the body so that they excreted fresh honeydew.
- Ants could also be observed transporting mealybugs, usually living individuals. This gives support to the theory that ants are able to move mealybugs on the grape to bring them to a better place for honeydew production. However, in this study, ants carried mealybugs back to the ant colony (presumably as prey, but we have no evidence to support this). Once ants tend a group of mealybugs for a few days, they become very possessive and will aggressively attack any intruder (even metal probes, small paint brushes, etc.).
- Ant activity also involves hygienic cleaning of the mealybug and its surroundings. The ants promptly removed the empty ovisacs and exuviae.

- The mealybug destroyer (small beetle) proved to be the best predator. Its larvae appear similar to mealybugs; however, they move quickly. The beetles feed on all mealybug stages, although small beetle larvae cannot feed on adult mealybugs because they are not able to eat through their waxy secretions. The adult beetles are very adept at moving into the waxy mealybug ovisac and feeding on eggs (killing hundreds!).

- Lacewing larvae are not as effective as mealybug destroyers. The small larvae have a difficult time moving into the wax secretion forming the mealybug ovisac. Although mealybugs, in the absence of ants, are relatively defenseless against predators, they excrete an "ostiolar fluid" when disturbed. This sticky fluid disrupted lacewing feeding and often dried on lacewing mouthparts, preventing feeding and, in some instances, resulting in the eventual death of the lacewing (starvation).

- *Pseudaphycus flavidulus* is a little wasp (less than 1mm), so the best observations were made below the microscope. Because of its' size, mealybugs do not seem to sense *P. flavidulus'*s presence, even when the parasitoid walks and antennate a mealybug for a long time before parasitizing it. *P. flavidulus* usually "stung" or oviposited in the side of the mealybug, where the wax secretion on the skin is easier to penetrate. Oviposition (egg laying) was relative quick. The parasite deposits 10-20 eggs per mealybug and prefers the larger mealybugs.

- *Leptomastix epona* is bigger than *P. flavidulus* (about 2mm) and appears to deposit a single egg per mealybug. *L. epona* also needed more time to antennate before oviposition (often more than 2 minutes). After oviposition the parasitoid did not fly away immediately; they continued to antennate the mealybug and forage nearby.

- In the presence of ants, the mealybug destroyer's appearance and behavior provided protection. In addition to their appearance, *C. montrouzieri* modified its behavior to model that of the mealybugs - in the vicinity of ants, they did not move and assumed a sedentary posture, like mealybugs. With this behavior, ants left the beetles alone; however, if the beetles were discovered moving very fast, ants quickly recognized them as an enemy and were able to kill them.

- Ants were not able to capture *P. flavidulus* very well (presumably because of the parasitoid's small size, quick oviposition, and rapid movement). However, when they detected the parasitoid near the mealybug they moved more rapidly and aggressively and often disrupted oviposition by *P. flavidulus*..

- Ants were better able to protect mealybugs from the slower moving, larger wasp (*Leptomastix epona*). Ants typically had direct confrontations with *L. epona* - sometimes the parasite was killed, more often the *L. epona* flew away.

Conclusions from the video are definitive: ants tending mealybugs milk them for honeydew and attempt to protect them from predators and parasitoids. In the small video arena, the ants were often successful in disrupting parasitoid oviposition. They were less successful in capturing the mealybug destroyer.

Part II Laboratory Exclusion Experiments: An enclosed system was used to test the influence of ants on the success of 2 parasitoid species. Colonies of the Argentine ant were housed in large plastic containers and reared in a similar manner as described for the native gray ant. The "foraging tube" was used to direct ants into small cages where mealybugs and parasitoids were housed. The mealybugs were reared on "half potatoes." (Potatoes were halved and the cut portion sealed with wax - this allowed the potato to be placed flush against a bottom surface and prevented mealybugs from hiding underneath the potato.) Tested potatoes were inoculated with a gravid female mealybug and held for 3 to 4 weeks while the eggs hatched and the mealybug population reached the second to third development stage (mealybugs were selectively removed to create uniform population densities). During this period, the potatoes were placed on 2 inch stands inside the cage, with the legs of half the stands covered with Tanglefoot to exclude ants. A Tanglefoot barrier ringed the inside base of the cage and prevented ants from foraging on the sides or top of the cage (ants foraged on the bottom). Therefore, the test arena placed parasitoids in a small arena with ants foraging on some potatoes and others without ants - parasitoids could choose where they searched for mealybugs.

Three separate cage trials were conducted: (1) *Leptomastix epona* (80 E, 30 G), (2) *Pseudaphycus flavidulus* (110 parasitoids - mostly E), (3) a mixed release of *Leptomastix epona* (40 E, 15 G) and *Pseudaphycus* (55 parasitoids - mostly E). After the release, populations of mealybugs and ants were checked periodically to note the number of parasitoids present and the interaction between insects. After all parasitoids were dead (about 3 weeks), individual potatoes were placed in canning jars and held for parasitoid emergence. After 4 weeks, the number of mealybugs from the original cohort were counted, with development stage and condition (live or parasitized) recorded.

Laboratory experiments are near completion, although the data have not yet been entered into the computer. However, initial observations can be made. In all trials, the ants were very actively tending the mealybugs and feed on the honeydew droplets on the potatoes and on the cage floor. In this enclosed system, the ants win the battle over the parasitoids. Observations indicate that when ants came in contact with parasitoids they would attempt and often succeed in catching and killing the small wasps. The parasitoids were killed not only on the "no exclusion" potatoes but when they rested on the cage bottom, sides or top - the ants foraged throughout the cage. Therefore, while the "exclusion" potato treatment offered a parasitoid refuge from ants, the small wasps are obviously not complex strategist and would eventually move into ant territory and be killed. For this reason, the parasitoid population would quickly declined inside the cages. There was greater percentage parasitism and lower mealybug numbers in the ant exclusion treatment.